[Proc. Roy. Soc. Victoria, 52 (N.S.), Pt. I., 1940.]

Art. VI.—*Observations on the Mineral and Vitamin Content of Australian Milk.*

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[From the Australian Institute of Anatomy, Canberra.]

[Read 13th July, 1939; issued separately, 1st March, 1940.]

Introduction.

The vitamin content of cow's milk has been shown to vary within reasonably wide limits, while it is well known that the mineral content, although remarkably constant, also varies within what might be termed physiological limits. During the routine analysis of a number of sweetened and unsweetened condensed milk samples from various parts of the world, Professor J. L. Rosedale, of the King Edward VII. College of Medicine in Singapore, found that the Australian samples were low in vitamin A. In a private communication he suggested that an explanation of the difference may be found in the reputedly low phosphorus content of Australian soils, and some support was given to this hypothesis by Richmond's (1930) suggestion that lecithin, because of its phosphorus content, may be an index of the vitamin (presumably vitamin A) content of butter-fat. Initially, the object of this investigation was to investigate Professor Rosedale's hypothesis and in so doing to determine the vitamin A content of a large number of Australian milk samples. Later, however, it was decided to extend the investigation with the object of discovering any correlations which might exist between the major vitamin and mineral constituents of milk.

One hundred and sixty-eight samples of mixed milk, representing the milk from 6,460 cows, were collected over a period of twelve months from New South Wales, Victoria and Tasmania. One hundred and twenty-five samples were known to be from afternoon milkings. The mineral constituents estimated were:—calcium, phosphorus, potassium, sodium, magnesium, sulphur, and iron. The vitamins estimated were:—vitamin A and carotene, vitamin B_1 (thiamin), vitamin B_2 (riboflavin), and vitamin C. The last named was estimated in each sample within twelve hours of collection and the milk was only exposed to direct daylight at the time of collection and while the estimation was being carried out in the laboratory. The methods employed for the estimation of the vitamin constituents were chosen because they involved the minimum amount of time and gave results, the relative values of which were reliable. But because the methods employed were non-biological the results are considered comparative only, although during preliminary work results obtained by these methods were checked against results obtained by well-controlled biological methods for the same samples and, so far as the results could be compared, they were in good agreement.

Methods of Analysis.

Fat was estimated by Richmond's (1930) modification of the Roese Gottlieb method. The specific gravity was determined with a Westphal balance and corrected for temperature.

VITAMIN A AND CAROTENE.

A pint of milk was centrifuged, the skim milk separated and kept for later determinations, the cream churned into butter, portion of the butter dissolved in petroleum ether and the whole transferred to a separating funnel containing distilled water. The ethereal layer was separated, washed and evaporated under suction at about 30°C. Because facilities were not available for estimating carotene spectro-photometrically, it was estimated in the warm residual butter-fat by a modification of Palmer's (1922) colorimetric method. Several workers have found that for butter-fat the graph given by Palmer gives carotene values which are several times too high, and Barnett (1934) has obtained a correction factor .28 which enables more accurate results to be obtained. However, Barnett assumes that carotene is the only colouring matter of consequence in butter-fat, but Gillam (1934) has shown that the ratio of carotene to xanthophyll in English butter-fat is fairly constant, being approximately 14 : 1 by weight, and has estimated the carotene value at approximately 94 per cent. of the total yellow colour of the butter-fat. This result is supported by the work of Baumann and Steenbock (1933) on an American butter-fat. Hence a more accurate conversion factor would be .263. Introducing this modification and simplifying Palmer's expression we have:—

$$\text{Percentage carotene} = \frac{.00268 \times .263 \times K_2Cr_2O_7 \text{ equivalent}}{\text{Depth of butter-fat}}$$

$$= \frac{\text{Reading on } x \text{ axis in fig. I}}{\text{Depth of butter-fat}}$$

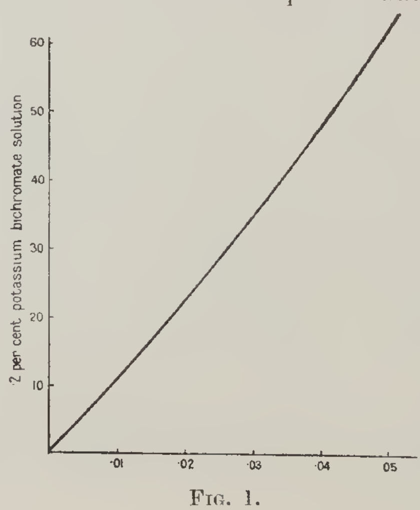

FIG. 1.

To estimate vitamin A, 2 gms. of butter-fat were saponified with 15 cc. of N/2 alcoholic potassium hydroxide, 20 ccs. of water added, the whole transferred to a separating funnel and extracted with two quantities of 25 ccs. of ether. The ethereal extracts were washed with water, then with N/2 aqueous potassium hydroxide and again with water, after which the ether was evaporated under suction at about 30°C. The residue was dissolved in purified ethyl alcohol, made up to 10 ccs. and vitamin A estimated in the solution with a Hilger Vitameter A using the factor 1600.

VITAMIN B_1.

In 1935, Schopfer published a paper showing that vitamin B_1 is a growth factor for the mould *Phycomyces blakesleeanus*, and he outlined a method for the estimation of vitamin B_1. It was later found (Robbins and Kavanagh (1937), Sinclair (1937), Schopfer and Jung (1937)) that both vitamin B_1 and its degradation products promote the growth of the mould. Hence the method about to be described for the estimation of vitamin B_1 actually estimates vitamin B_1 and any of its breakdown products which might be present in fresh milk.

Into 50 cc. Erlenmeyer flasks were placed 0.2 cc. of skim milk, each milk sample to be analysed, being done in triplicate. Skim milk was used, for otherwise a thin layer of butter-fat settled on the surface of the media and the slight anaerobic conditions thus introduced inhibited the growth of the mould. To each flask was then added 10 ccs. of a medium consisting of :—

Glucose	166.8	gms.
Asparagin	6.4	,,
$MgSO_4 + 7H_2O$	0.84	,,
KH_2PO_4	2.5	,,
H_2O	1,665	ccs.

and the pH was adjusted to approximately 6.6 by the addition of one or two drops of dilute sodium hydroxide.

A standard range was also set up containing 10 ccs. of media as before, but in place of milk the following amounts of vitamin B_1 were added:—0.5, 0.4, 0.3, 0.2, 0.1, 0.01, 0.001 international units. These flasks were set up in duplicate and all the flasks then plugged and sterilized at 107°C. for 10 minutes. A culture of *Phycomyces blakesleeanus* was prepared a fortnight previously in 100 ccs. of Wort Agar, made as follows:—25 ccs. of malt extract, 4 gms. of agar, and 200 ccs. of water were made up and autoclaved at 110°C. for 15 minutes. Several grams of the spore bearing mycelium were removed with sterile forceps and thoroughly washed in sterile water contained in a beaker covered with a watch glass. Each flask was then inoculated with two drops (about 0.2 cc.) of the spore bearing suspension by means of a sterile pipette. The suspension was stirred frequently to ensure that the spores did not sediment. The inoculated flasks were then left in the dark at room temperature (22°C.) for ten days. All the mycelia were then removed, washed in running water, alcohol, and ether, rolled into small balls, dried in a hot air oven at 110°C. and then weighed.

From the weights of the mycelia in the standard flasks, a graph, of which Fig. II. is typical, was drawn, and from this graph the amounts of vitamin B_1 in the other flasks calculated.

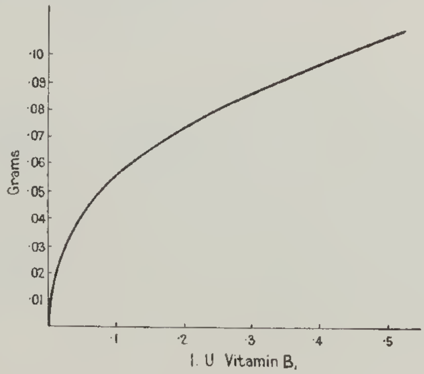

Fig. 2.

Vitamin B_2.

Was estimated in 100 ccs. of skim milk according to the second method described by Weisberg and Levin (1937). The aqueous solution of riboflavin contained much foreign matter, but this did not interfere with the estimation. The relation between the fluorescence intensity of sodium fluorescein (May and Baker Limited) and vitamin B_2 is given in Fig. III., which differs slightly from that given by Weisberg and Levin, for it is a straight line which does not pass through the origin.

Vitamin C.

To 10 ccs. of milk were added 10 ccs. of 20 per cent. trichloracetic acid; the solution was mixed well and then filtered. The precipitate was washed once with a little distilled water and the filtrate made up to 25 ccs. A burette was charged with this solution and it was added drop by drop to a standardized solution of 2:6 dichlorphenolindophenol until the red colour was just discharged. Knowing the strength of the dichlorphenolindophenol solution, the vitamin C present was determined.

Ashing.

300 ccs. of milk in a porcelain basin were dried on a water bath, a little alcohol being added to facilitate evaporation. When dry, the residue was heated over a bunsen to set the carbon and then placed in a muffle at a temperature of 400°–450°C. To

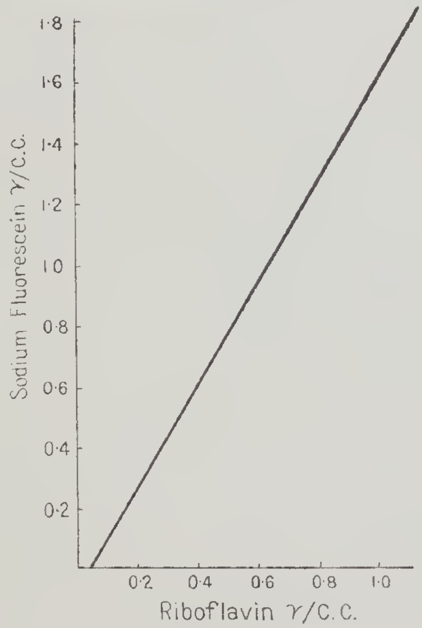

FIG. 3.

complete the incineration, the carbonized mass was cooled, mixed with water, dried, and returned to the muffle. The following determinations were carried out in duplicate on the ash which was dissolved in the minimum amount of hydrochloric acid and the volume made up to 100 ccs.:—

1. *Calcium.*—25 ccs. of the ash solution were made alkaline with ammonia and the resulting precipitate filtered off and washed. The filtrate and washings were kept for the sulphur determination. The precipitate was then dissolved in the minimum amount of hydrochloric acid, sodium acetate solution added and adjustment made to pH 5.0, using congo red as indicator. Calcium was then precipitated at 40°C. with an excess of saturated ammonium oxalate, the solution allowed to stand one hour, filtered, washed with water and a little dilute ammonia, incinerated and weighed as CaO.

2. *Magnesium.*—The combined filtrate and washings were evaporated to about 100 ccs., and 20 ccs. of nitric acid added. The beaker was covered, evaporated to dryness, 5 ccs. of hydrochloric acid were added, then evaporated almost to dryness and the residue dissolved in hot water. The magnesium was precipitated by the addition of 3 ccs. of a 10 per cent. solution of ammonium phosphate and sufficient ammonium hydroxide to make the solution slightly alkaline. The solution was stirred vigorously, allowed to stand 15 minutes, 15 ccs. of ammonium hydroxide added and the precipitation allowed to proceed over-

night. It was then filtered, washed with dilute ammonia, transferred to a crucible, dried, ignited, and weighed as $Mg_2P_2O_7$.

3. *Sulphur.*—The filtrate from the calcium estimation to which 10 ccs. of concentrated hydrochloric acid had been added was evaporated almost to dryness. It was then made up to 250 ccs., 5 ccs. of dilute hydrochloric acid added, and boiled. Some acidified 3 per cent. barium chloride was also boiled and 8 ccs. slowly added to the sulphate solution. It was left for an hour, filtered, washed, ignited, cooled, three drops of a mixture containing 1 cc. of alcohol and 2 ccs. of sulphuric acid added, incinerated and weighed as $BaSO_4$.

4. *Potassium.*—To the filtrate from the sulphate determination, barium hydroxide solution was added in slight excess and it was evaporated to about 50 ccs. Ammonium carbonate was added, the solution filtered into a porcelain dish, washed with hot water and evaporated to dryness. Moisture and ammonium chloride were driven off, 5 ccs. of hot water added, the solution filtered into a tared beaker, the residue washed, one drop of hydrochloric acid added, the filtrate evaporated to dryness and weighed as $NaCl + KCl$. 15 ccs. of 20 per cent. perchloric acid were added and it was evaporated almost to dryness. To the almost dry, cold residue 10 ccs. of a washing solution consisting of 1 cc. of 20 per cent. perchloric acid in 100 ccs. of 98 per cent. alcohol were added. The solution was stirred well, set aside for 5 minutes, decanted through a tared sintered glass crucible, placed on the steam bath to remove alcohol, dissolved in the minimum amount of hot water, evaporated, 10 ccs. of washing solution added, stirred, filtered, dried for one hour at 130°C. and weighed as $KClO_4$.

5. *Sodium* was calculated from the two above results by difference.

6. *Phosphorus.*—The original solution was again made up to 100 ccs. and to 15 ccs. a little methyl orange was added and the solution boiled to drive off carbon dioxide. The solution was neutralized with sodium hydroxide, 25 ccs. of 10 per cent. calcium chloride and a few drops of phenolphthalein added and the solution titrated with N/10 sodium hydroxide until a slight pink remained permanent after mixing. The percentage phosphorus was given by—

$$\frac{\text{cc. N/10 NaOH} \times .1551}{\text{weight of milk taken.}}$$

7. *Iron.*—To 35 ccs. of the remaining ash solution diluted to 100 ccs. were added 10 ccs. of concentrated hydrochloric acid. The iron was precipitated with 4 per cent. cupferron, filtered under suction, washed with water, dilute ammonia and again with water. The precipitate was transferred to an annealing cup, heated over a bunsen, incinerated and weighed directly as Fe_2O_3.

Results.

The results of the analyses are given in Tables I. and II.

TABLE I.—(MINERAL CONSTITUENTS.)

Number.	Specific Gravity.	Percentage Ca.	Percentage P.	Percentage K.	Percentage Na.	Percentage Mg.	Percentage S.	Percentage Fe.
1 ..	1·0315	·130	·082	·156	·044	·011	·009	·000090
2 ..	1·0320	·125	·088	·148	·042	·009	·010	·000108
3 ..	1·0310	·120	·086	·142	·042	·012	·009	·000072
4 ..	1·0332	·107	·087	·134	·048	·011	·008	·000081
5 ..	1·0331	·134	·090	·145	·051	·009	·013	·000126
6 ..	1·0330	·118	·097	·148	·050	·013	·008	·000081
7 ..	1·0321	·122	·094	·142	·049	·014	·008	..
8 ..	1·0325	·111	·090	·138	·051	·011	·007	..
9 ..	1·0320	·120	·086	·140	·057	·010	·008	·000108
10 ..	1·0317	·118	·075	·145	·054	·013	·008	·000108
11 ..	1·0315	·125	·091	·152	·045	·014	·010	·000065
12 ..	1·0317	·128	·086	·150	·040	·012	·010	·000073
13 ..	1·0310	·127	·078	·146	·042	·011	·011	·000068
14 ..	1·0315	·140	·084	·150	·045	·013	·016	·000084
15 ..	1·0311	·119	·080	·144	·047	·012	·009	·000104
16 ..	1·0320	·125	·085	·147	·050	·011	·009	·000070
17 ..	1·0311	·130	·084	·143	·045	·012	·010	·000081
18 ..	1·0310	·135	·087	·150	·050	·011	·012	·000210
19 ..	1·0311	·116	·079	·135	·058	·009	·009	·000089
20 ..	1·0312	·125	·087	·142	·053	·012	·009	·000090
21 ..	1·0310	·116	·082	·140	·048	·009	·008	·000075
22 ..	1·0316	·117	·083	·143	·050	·010	·009	·000080
23 ..	1·0315	·112	·080	·145	·049	·011	·008	·000099
24 ..	1·0311	·130	·084	·148	·051	·011	·010	·000088
25 ..	1·0305	·126	·080	·146	·040	·010	·009	·000074
26 ..	1·0314	·125	·079	·146	·043	·011	·007	·000081
27 ..	1·0311	·122	·075	·151	·041	·011	·008	·000086
28 ..	1·0311	·126	·081	·147	·042	·011	·009	·000109
29 ..	1·0310	·125	·079	·147	·049	·009	·009	·000079
30 ..	1·0310	·122	·079	·139	·045	·011	·008	·000063
31* ..	1·0317	·097	·082	·125	·046	·007	·007	·000062
32 ..	1·0304	·117	·077	·138	·060	·011	·008	·000084
33 ..	1·0304	·124	·075	·140	·055	·011	·009	·000088
34 ..	1·0313	·110	·078	·139	·048	·010	·009	·000078
35 ..	1·0307	·120	·082	·145	·049	·014	·009	·000090
36 ..	1·0312	·123	·080	·150	·045	·007	·009	·000067
37 ..	1·0305	·112	·082	·145	·041	·010	·008	·000080
38 ..	1·0314	·121	·070	·151	·042	·009	·008	..
39* ..	1·0303	·079	·076	·147	·045	·011	·008	·000092
40 ..	1·0312	·126	·075	·153	·046	·010	·010	·000105
41 ..	1·0314	·119	·081	·142	·047	·011	·008	·000096
42 ..	1·0304	·119	·076	·148	·050	·012	·008	·000065
43 ..	1·0319	·131	·082	·154	·046	·011	·010	..
44 ..	1·0314	·126	·078	·153	·053	·011	·009	·000101
45* ..	1·0300	·117	·080	·145	·040	·010	·008	·000068
46 ..	1·0310	·119	·081	·148	·050	·011	·009	·000067
47 ..	1·0315	·080	·074	·136	·051	·011	·009	·000067
48* ..	1·0319	·134	·092	·145	·048	·010	·012	·000070
49* ..	1·0310	·120	·078	·148	·047	·010	·009	..
50 ..	1·0299	·112	·077	·139	·050	·011		..
51 ..	1·0306	·125	·085	·151	·053	·013		..
52 ..	1·0313	·118	·0 0	·148	·048	·011	·009	..
53 ..	1·0298	·135	·079	·157	·058	·019	·012	..
54* ..	1·0320	·122	·088	·156	·050	·013	·009	..
55 ..	1·0323	·111	·081	·152	·046	·011	·007	..
56 ..	1·0320	·116	·080	·150	·051	·012	·009	..
57 ..	1·0301	·095	·079	·142	·041	·012	·008	..
58 ..	1·0300	·116	·080	·148	·040	·011	·009	..
59 ..	1·0298	·102	·080	·147	·042	·010	·008	..
60 ..	1·0316	·128	·086	·149	·041	·010	·009	..
61 ..	1·0306	·120	·081	·148	·040	·011	·009	..
62 ..	1·0313	·123	·078	·151	·042	·011	·009	..
63 ..	1·0316	·126	·080	·150	·041	·010	·008	..
64* ..	1·0318	·128	·088	·148	·055	·011	·012	..
65* ..	1·0316	·108	·080	·139	·052	·011	·009	..
66 ..	1·0324	·118	·089	·142	·049	·012	·009	..

TABLE I.—(MINERAL CONSTITUENTS)—*continued.*

Number.	Specific Gravity.	Percentage Ca.	Percentage P.	Percentage K.	Percentage Na.	Percentage Mg.	Percentage S.	Percentage Fe.
67	1·0323	·110	·084	·144	·047	·009	·008	..
68*	1·0310	·131	.081	·152	·046	·011	·011	..
69*	1·0316	·114	·085	·148	·047	·011	·008	..
70*	1·0317	·135	·082	·154	·051	·016	·011	..
71*	1·0316	·119	·080	·148	·018	·011	·009	..
72	1·0295	·108	·080	·146	·044	·010	·009	..
73*	1·0300	·127	·086	·150	·012	·011	·009	..
74*	1·0308	·130	·088	·154	·048	·013	·011	..
75	1·0330	·111	·080	·148	·049	·011	·007	..
76	1·0317	·118	·082	·145	·050	·011	·007	..
77	1·0316	·125	·084	·148	·052	·010	·008	..
78	1·0333	·130	·081	·151	·051	·011	·010	..
79	1·0321	·125	·080	·144	·056	·011	·009	..
80	1·0328	·121	·079	·143	·051	·011	·010	..
81	1·0320	·133	·080	·147	·049	·015	·010	..
82	1·0338	·121	·086	·143	·045	·013	·009	..
83	1·0316	·119	·082	·138	·051	·010	·009	..
84	1·0308	·122	·089	·127	·041	·011	·007	..
85	1·0307	·124	·078	·130	·039	·010	·008	..
86	1·0321	·099	·080	·119	·039	·009	·006	..
87	1·0315	·122	·081	·134	·043	·011	·009	..
88	1·0309	·123	·084	..	..	·011	·006	..
89	1·0303	·127	·086	·130	·039	·010	·004	..
90	1·0310	·135	·088	..	..	..	..	..
91	1·0302	·129	·083	..	..	·012	·003	..
92*	1·0308	·120	·083	·140	·053	·011	·007	..
93*	1·0319	·124	·088	·152	·055	·012	·008	..
94	1·0308	·114	·080	·138	·054	·012	·007	..
95*	1·0319	·119	·089	·136	·015	·011	·007	..
96	1·0320	·130	·086	·143	·048	·011	·010	..
97	1·0315	·121	·085	·148	·045	·009	·011	..
98*	1·0313	·094	·074	·140	·044	·013	·009	..
99*	1·0311	·123	·081	·149	·052	·008	·010	..
100	1·0317	·124	·087	·156	·048	·011	·009	..
101	1·0318	·129	·088	·150	·050	·012	·009	..
102	1·0319	·127	·084	·146	·051	·010	·008	..
103	1·0321	·105	·084	·132	·017	·012	·007	..
104	1·0312	·122	·074	·138	·042	·011	·008	..
105	1·0309	·121	·078	·143	·052	·010	·008	..
106	1·0308	·120	·082	·146	·018	·011	·009	..
107	1·0317	·132	·078	·156	·046	·012	·009	..
108	1·0317	·120	·080	·153	·044	·011	·008	..
109	1·0312	·124	·079	·146	·045	·009	·098	..
110	1·0315	·129	·079	·150	·048	·012	·008	..
111	1·0314	·120	·082	·138	·055	·011	·007	..
112	1·0313	·114	·081	·142	·039	·013	·007	..
113	1·0319	·120	·072	·140	·048	·010	·012	..
114	1·0328	·135	·088	·154	·045	·013	·010	..
115	1·0319	·124	·079	·134	·050	·011	·009	..
116	1·0318	·134	·083	·138	·049	·011	·099	..
117	1·0314	·129	·085	·140	·050	·011	·012	..
118	1·0315	·114	·082	·128	·056	·011	·008	..
119	1·0312	·132	·092	·145	·052	·012	·011	..
120	1·0322	·130	·085	·142	·054	·014	·010	..
121	1·0312	·126	·075	·135	·045	·011	·010	..
122	1·0313	·124	·087	·138	·045	·013	·011	..
123	1·0324	·127	·086	·135	·046	·011	·009	..
124	1·0329	·121	·073	·138	·048	·009	·098	..
125	1·0318	·109	·076	·124	·048	·010	·008	..
126	1·0317	·116	·074	·130	·046	·010	·007	..
127	1·0314	·117	·080	·148	·047	·012	·010	..
128	1·0304	·112	·079	·145	·042	·009	·010	..
129	1·0311	·122	·084	·150	·048	·012	·009	..
130	1·0312	·119	·086	·146	·049	·011	·008	..
131	1·0324	·125	·078	·148	·052	·012	·007	..
132	1·0314	·102	·066	·138	·041	·013	·006	..
133	1·0309	·121	·085	·141	·050	·011	·008	..
134	1·0314	·120	·080	·140	·048	·011	·008	..
135	1·0303	·119	·079	·143	·046	·010	·010	..
136	1·0309	·114	·077	·141	·058	·011	·007	..
137	1·0325	·129	·077	·153	·056	·013	·009	..

TABLE I.—(MINERAL CONSTITUENTS)—*continued.*

Number.	Specific Gravity.	Percentage Ca.	Percentage P.	Percentage K.	Percentage Na.	Percentage Mg.	Percentage S.	Percentage Fe.
138 ..	1·0329	·121	·084	·149	·040	·011	·008	..
139 ..	1·0319	·105	·075	·147	·045	·008	·007	..
140 ..	1·0329	·114	·086	·148	·050	·012	·008	..
141 ..	1·0316	·094	·078	·119	·045	·011	·007	..
142 ..	1·0313	·118	·074	·134	·048	·010	·012	..
143 ..	1·0318	·097	·078	·145	·050	·008	·009	..
144 ..	1·0311	·110	·082	·132	·038	·009	·009	..
145 ..	1·0323	·118	·078	·131	·056	·011	·010	..
146 ..	1·0320	·119	·082	·137	·052	·010	·012	..
147 ..	1·0317	·108	·089	·141	·054	·010	·008	..
148 ..	1·0301	·140	·085	·158	·050	·011	·009	..
149 ..	1·0308	·126	·083	·153	·050	·011	·008	..
150 ..	1·0300	·123	·082	·151	·042	·012	·007	..
151 ..	1·0326	·125	·089	·147	·046	·011	·008	..
152 ..	1·0323	·117	·084	·148	·050	·012	·007	..
153 ..	1·0326	·110	·080	·144	·048	·011	·010	..
154 ..	1·0318	·095	·071	·135	·049	·010	·010	..
155 ..	1·0313	·112	·078	·132	·042	·011	·009	..
156 ..	1·0310	·121	·081	·134	·048	·011	·010	..
157 ..	1·0309	·131	·087	·147	·042	·011	·011	..
158 ..	1·0317	·133	·078	·146	·050	·014	·012	..
159 ..	1·0312	·121	·078	·142	·053	·010	·009	..
160 ..	1·0321	·125	·080	·150	·044	·011	·008	..
161 ..	1·0315	·114	·076	·141	·046	·011	·009	..
162 ..	1·0316	·126	·079	·138	·048	·012	·010	..
163 ..	1·0315	·128	·078	·154	·052	·011	·009	..
164 ..	1·0315	·119	·089	·150	·054	..	·008	..
165 ..	1·0311	·135	·079	·157	·056	·011	·014	..
166 ..	1·0318	·116	·078	·148	·048	·011	·009	..
167 ..	1·0311	·129	·082	·145	·046	·011	·010	..
168 ..	1·0316	·120	·081	·139	·048	·011	·009	..
Mean ..	1·0318†	·120	·082	·144	·048	·012	·009	·000087

† See text.

TABLE I.—(VITAMIN CONSTITUENTS.)

Number.	Percentage Fat.	Percentage Carotene in B.F.	I.U. Vitamin A /gm. B.F.	I.U. Vitamin B_1 /100 ccs.	Mgms. Vitamin B_2 /100 ccs.	Percentage Vitamin C.
1 ..	4·37	·00082	51	19	·22	·00260
2 ..	4·53	·00036	48	25	·17	·00226
3 ..	4·55	·00070	48	17	·12	·00080
4 ..	4·95	·00048	54	20	·22	·00034
5 ..	4·14	·00060	53	24	·17	·00044
6 ..	3·95	·00105	35	22	·16	·00036
7 ..	4·75	·00059	33	20	·18	·00074
8 ..	5·35	·00076	35	15	·12	·00186
9 ..	3·20	·00029	40	20	·12	·00046
10 ..	4·25	·00062	41	18	·17	·00200
11 ..	4·15	·00071	68	20	·16	·00048
12 ..	5·55	·00110	52	15	·15	·00180
13 ..	5·20	·00054	54	19	·19	·00250
14 ..	5·15	·00125	56	10	·17	·00178
15 ..	5·05	·00136	29	20	·19	·00079
16 ..	4·43	·00096	57	21	·20	·00087
17 ..	6·20	·00238	54	19	·12	·00128
18 ..	5·10	·00119	44	19	·16	·00230
19 ..	4·78	·00110	35	18	·17	·00198
20 ..	4·40	·00065	36	20	·15	·00290
21 ..	4·10	·00031	33	17	·14	·00052
22 ..	4·37	·00078	34	24	·12	·00248
23 ..	5·62	·00102	28	14	·16	·00032
24 ..	5·40	·00068	36	14	·15	·00094
25 ..	4·60	·00085	40	20	·20	·00224
26 ..	4·25	·00096	50	18	·14	·00108

TABLE I.—(VITAMIN CONSTITUENTS)—*continued.*

Number.	Percentage Fat.	Percentage Carotene in B.F.	I.U. Vitamin A /gm. B.F.	I.U. Vitamin B_1 /100 ccs.	Mgms. Vitamin B_2 /100 ccs.	Percentage Vitamin C.
27 ..	4·10	·00075	66	15	·22	·00056
28 ..	5·22	·00062	61	22	·16	·00224
29 ..	4·65	·00059	29	18	·15	·00240
30 ..	4·48	·00046	31	19	·14	·00054
31 ..	4·05	·00031	58	10	·18	·00032
32 ..	5·45	·00112	67	19	·17	·00044
33 ..	5·10	·00107	62	17	·16	·00034
34 ..	5·05	·00113	46	20	·17	·00034
35 ..	5·05	·00155	61	19	·15	·00050
36 ..	4·23	·00036	53	19	·19	·00032
37 ..	6·10	·00204	38	18	·18	·00048
38 ..	5·10	·00118	45	25	·16	·00258
39 ..	4·58	·00075	38	20	·18	·00303
40 ..	3·92	·00084	45	20	·12	·00240
41 ..	4·67	·00112	56	24	·15	·00172
42 ..	4·30	·00058	55	18	·19	·00192
43 ..	4·00	·00084	66	19	·16	·00206
44 ..	4·47	·00078	50	22	·21	·00192
45 ..	5·52	·00153	60	15	·19	·00048
46 ..	5·30	·00062	36	18	·21	·00254
47 ..	4·20	·00070	40	15	·16	·00224
48 ..	4·50	·00093	40	21	·18	·00192
49 ..	4·15	·00103	45	14	·17	·00234
50 ..	4·00	·00142	33	14	·21	·00172
51 ..	5·12	·00094	37	15	·16	·00238
52 ..	4·55	·00090	34	18	·21	·00168
53 ..	4·18	·00189	41	17	·12	·00188
54 ..	3·98	·00088	56	19	·21	·00184
55 ..	4·45	·00133	57	17	·26	·00220
56 ..	3·85	·00145	68	19	·18	·00160
57 ..	4·93	·00148	74	17	·22	·00046
58 ..	4·65	·00078	52	19	·17	·00192
59 ..	4·75	·00115	54	15	·16	·00101
60 ..	4·90	·00138	63	19	·25	·00186
61 ..	5·62	·00165	70	17	·21	·00238
62 ..	3·65	·00109	60	18	·17	·00182
63 ..	3·50	·00158	62	20	·12	·00196
64 ..	4·05	·00045	59	19	·22	·00192
65 ..	3·95	·00150	66	18	·24	·00190
66 ..	5·00	·00116	56	15	·12	·00224
67 ..	4·50	·00148	51	18	·18	·00122
68 ..	4·70	·00148	55	19	·19	·00184
69 ..	5·15	·00206	72	15	·22	·00252
70 ..	4·90	·00228	44	18	·23	·00182
71 ..	5·73	·00058	38	25	·17	·00184
72 ..	4·80	·00190	52	21	·18	·00162
73 ..	6·75	·00119	55	20	·19	·00240
74 ..	6·00	·00228	40	14	·22	·00123
75 ..	3·80	·00107	33	20	·17	·00250
76 ..	4·55	·00125	45	24	·12	·00222
77 ..	3·60	·00119	36	18	·18	·00062
78 ..	3·43	·00141	49	14	·18	·00108
79 ..	3·15	·00138	39	15	·13	·00188
80 ..	4·00	·00145	35	18	·18	·00092
81 ..	3·40	·00174	67	14	·20	·00156
82 ..	5·30	·00242	45	17	·19	·00120
83 ..	3·83	·00135	38	19	·17	·00098
84 ..	3·95	·00070	33	17	·12	·00250
85 ..	3·80	·00183	37	20	·12	·00222
86 ..	3·45	·00098	40	21	·14	·00148
87 ..	4·25	·00135	47	19	·20	·00260
88 ..	3·85	·00111	29	17	·09	·00306
89 ..	3·65	·00167	34	19	·17	·00150
90 ..	3·70	·00060	35	19	·18	·00054
91 ..	3·90	·00245	33	20	·27	·00044
92 ..	5·00	·00090	46	..	·17	·00096
93 ..	4·60	·00078	46	24	·17	·00084
94 ..	4·40	·00081	36	19	·18	·00093
95 ..	5·15	·00101	45	16	·17	·00139
96 ..	..	·00098	47	19	·20	·00200
97 ..	..	·00105	68	18	·19	·00098
98 ..	4·12	·00065	52	20	·16	·00192

TABLE I.—(VITAMIN CONSTITUENTS)—*continued.*

Number.	Percentage Fat.	Percentage Carotene in B.F.	I.U. Vitamin A /gm. B.F.	I.U. Vitamin B_1 /100 ccs.	Mgms. Vitamin B_2 /100 ccs.	Percentage Vitamin C.
99 ..	3·98	·00058	44	25	·13	·00128
100 ..	..	·00019	29	20	·16	·00130
101 ..	4·33	·00155	56	18	·17	·00084
102 ..	5·34	·00134	44	24	·14	·00238
103 ..	4·88	·00221	54	19	·16	·00156
104 ..	4·28	·00190	57	23	·20	·00044
105 ..	4·27	·00105	33	18	·12	·00036
106 ..	4·63	·00045	34	15	·19	·00172
107 ..	4·35	·00056	35	28	·16	·00068
108 ..	4·85	·00095	28	20	·13	·00034
109 ..	4·04	·00060	36	18	·17	·00036
110 ..	3·85	·00058	36	18	·15	·00206
111 ..	4·65	·00050	40	15	·19	·00044
112 ..	5·25	·00161	68	19	·30	·00042
113 ..	3·10	·00172	61	21	·12	·00302
114 ..	4·15	·00121	56	14	·15	·00117
115 ..	4·05	·00150	60	14	·15	·00192
116 ..	5·45	·00065	40	19	·16	·00151
117 ..	5·10	·00084	37	15	·18	.
118 ..	5·05	·00120	33	18	·17	·00192
119 ..	4·05	·00114	38	17	·17	·00166
120 ..	4·33	·00074	45	26	·17	·00086
121 ..	6·10	·00036	67	14	·20	·00268
122 ..	5·00	·00018	35	23	·16	·00192
123 ..	4·68	·00056	38	19	·18	·00258
124 ..	4·30	·00098	57	20	·28	·00136
125 ..	4·00	·00145	62	22	·17	·00248
126 ..	4·90	·00200	46	23	·18	·00220
127 ..	5·63	·00068	61	13	·15	·00068
128 ..	4·90	·00064	35	24	·19	·00232
129 ..	6·45	·00079	39	25	·22	·00240
130 ..	6·10	·00082	49	27	·20	·00168
131 ..	3·70	·00067	45	22	·16	·00210
132 ..	4·65	·00104	36	18	·16	·00254
133 ..	3·50	·00092	53	16	·15	·00224
134 ..	3·53	·00040	38	20	·14	·00322
135 ..	3·05	·00084	45	21	·17	..
136 ..	4·10	·00112	28	17	·19	..
137 ..	3·30	·00084	45	16	·18	..
138 ..	5·20	·00058	56	15	·23	..
139 ..	3·73	·00065	55	23	·16	..
140 ..	3·85	·00153	66	25	·15	..
141 ..	3·42	·00078	33	15	·17	..
142 ..	4·25	·00103	40	22	·17	..
143 ..	3·75	·00093	35	19	·29	..
144 ..	3·45	·00070	52	20	·22	..
145 ..	3·70	·00090	38	21	·23	..
146 ..	3·95	·00102	44	14	·15	..
147 ..	5·15	·00132	62	16	·22	..
148 ..	4·12	·00145	55	20	·17	..
149 ..	3·98	·00148	51	22	·18	..
150 ..	4·33	·00165	56	18	·15	..
151 ..	5·34	·00109	50	24	·19	..
152 ..	4·88	·00058	60	22	·14	..
153 ..	4·28	·00070	36	15	·19	..
154 ..	4·27	·00107	40	18	·17	..
155 ..	4·63	·00086	30	15	·17	..
156 ..	5·05	·00075	45	18	·18	..
157 ..	5·10	·00084	56	19	·22	..
158 ..	4·20	·00078	66	25	·17	..
159 ..	4·75	·00068	59	15	·14	..
160 ..	3·90	·00070	62	11	·19	..
161 ..	4·10	·00090	60	18	·14	..
162 ..	4·00	·00135	60	20	·10	..
163 ..	4·65	·00111	63	25	·18	..
164 ..	5·20	·00167	54	21	·19	..
165 ..	4·85	·00063	62	20	·19	..
166 ..	3·90	·00065	51	14	·13	..
167 ..	4·20	·00058	65	19	·18	..
168 ..	4·06	·00079	43	20	·17	..
Mean ..	4·51	·00104	47·6	18·8	·17	·00156

General Discussion of Results.

In Table I. the mean specific gravity was not obtained by summing all the specific gravities and dividing by their number. To average specific gravities they should be first calculated to specific volumes, these averaged, and the average specific gravity deduced from the average specific volume. The average specific gravity then was calculated from the following:—

$$S.G. = \cfrac{1}{\cfrac{\frac{1}{1.0315} + \frac{1}{1.0320} + \frac{1}{1.0310} + \cdots\cdots + \frac{1}{1.0316}}{168}}$$

When the specific gravity and fat content of milk are known, the total solids and solids-not-fat may be readily calculated by means of Richmond's (1930) milk scale. The mean total solids and solids-not-fat content of the milk samples estimated in this manner are 13.50 and 8.99 per cent. respectively.

A slight direct correlation was found to exist between the solids-not-fat and phosphorus content of the samples, the Pearsonian coefficient of correlation being .40.

In Table III. the composition of milk from different breeds of cattle including the Australian Illawarra Shorthorn is compared. It will be seen that the composition of milk from Australian Illawarra Shorthorn herds resembles that from Ayrshire rather than Guernsey or Jersey herds. All samples were from afternoon milkings.

Table III.—Composition of Milk from Cows of Different Breeds.

Breed.	Number of Samples.	Total Solids Per Cent.			Fat Per Cent.			Solids-not-fat Per Cent.		
		Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.
		%	%	%	%	%	%	%	%	%
Australian Illawarra Shorthorn	16	14·32	11·67	13·02	4·93	3·35	4·35	9·39	8·32	8·67
Ayrshire ..	12	14·10	11·94	13·18	5·05	3·50	4·43	9·05	8·44	8·75
Guernsey ..	4	14·81	14·36	14·56	5·45	5·30	5·37	9·36	9·06	9·19
Jersey ..	10	15·53	13·04	14·25	6·10	4·50	5·36	9·43	8·54	8·89

Vitamin A and Carotene.

Very few figures have been published on the vitamin A content of butter-fat which are comparable with those obtained during this investigation. In the large majority of cases the estimations have been carried out on butter-fat from a small number of cows, a particular breed of cow, or on butter-fat obtained during a

TABLE II.—FREQUENCY DISTRIBUTION OF RESULTS.

																				Mean
Specific Gravity D.	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314
F.	1	0	0	2	1	4	2	1	1	4	2	3	2	6	6	11	11	9	8	9
Fat .. D.	..	..	..	..	..	3·1	3·2	3·3	3·4	3·5	3·6	3·7	3·8	3·9	4·0	4·1	4·2	4·3	4·4	4·5
F.	..	..	..	..	..	2	2	1	3	5	1	6	4	8	13	11	8	13	6	6
Carotene .. D	..	..	..	..	..	..	..	30	36	42	48	54	60	66	72	78	84	90	96	102
F.	..	..	..	..	..	..	..	3	2	1	6	3	16	11	8	12	12	6	9	6
Vitamin A .. D.	..	..	..	..	..	..	..	..	..	28	30	32	34	36	38	40	42	44	46	48
F.	..	..	..	..	..	..	..	..	..	3	5	1	13	17	11	12	2	6	15	4
Vitamin B_1 D.	..	..	..	..	..	..	..	..	..	..	10	11	12	13	14	15	16	17	18	19
F.	..	..	..	..	..	..	..	..	..	..	2	1	0	1	12	18	4	12	25	28
Vitamin B_2 D.	..	..	..	..	..	..	..	..	..	..	..	9	10	11	12	13	14	15	16	17
F.	..	..	..	..	..	..	..	..	..	..	..	1	1	0	14	4	9	14	20	31
Vitamin C .. D.	..	..	..	..	36	44	52	60	68	76	84	92	100	108	116	124	132	140	148	156
F.	..	..	..	..	10	8	7	2	2	2	5	3	4	2	1	3	3	2	3	1
Calcium .. D.	< 100	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
F.	9	2	0	0	2	0	1	3	1	4	3	5	0	7	0	6	5	7	11	10
Phosphorus.. D	..	..	..	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82
F.	..	..	..	1	0	0	0	1	1	1	1	5	6	4	4	17	14	23	12	16
Potassium .. D.	< 126	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144
F.	4	0	1	1	0	3	1	3	0	5	4	2	1	11	5	8	4	9	6	4
Sodium .. D.	..	..	..	..	..	..	..	..	..	38	39	40	41	42	43	44	45	46	47	48
F.	..	..	..	..	..	..	..	..	..	1	4	6	7	13	2	5	15	12	7	22
Magnesium D.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	07	08	09	10	11	12
F.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2	3	13	28	75	24
Sulphur .. D	..	..	..	..	..	..	..	..	..	..	..	..	..	..	04	05	06	07	08	09
F.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	0	4	20	43	53
Iron .. D.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	060	065	070	075	080	085
F.	..	..	..	..	..	..	..	..	..	..		..	..	..	1	6	5	3	8	3

Specific Gravity D.	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	..	..	..
F.	12	11	11	7	8	8	5	1	4	3	2	2	0	2	3	2	1	2	1	..	..	..
Fat .. D.	4·6	4·7	4·8	4·9	5·0	5·1	5·2	5·3	5·4	5·5	5·6	5·7	5·8	5·9	6·0	6·1	6·2	6·3	6·4	6·5	< 6·6	..
F.	8	8	5	9	5	11	8	5	2	3	4	1	0	0	1	3	1	0	0	1	1	..
Carotene .. D	108	114	120	126	132	138	144	150	156	162	168	174	180	186	192	198	-04	210	216	222	228	< 234
F.	10	9	6	2	3	6	6	6	5	1	4	2	0	1	3	1	2	0	0	1	2	3
Vitamin A .. D.	50	52	54	56	58	60	62	64	66	68	70	72	74	..	..	..	..	..	..	..	..	..
F.	5	9	9	14	5	8	10	3	6	7	1	1	1	..	..	..	..	..	..	..	..	..
Vitamin B_1 D.	20	21	22	23	24	25	26	27	28	..	..	..	..	..	..	..	..	..	..	..	..	..
F.	25	8	8	4	8	8	1	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..
Vitamin B_2 D.	18	19	20	21	22	23	24	25	26	27	28	29	30	..	..	..	..	..	..	..	..	..
F.	21	18	8	6	11	3	1	1	1	1	1	1	1	..	..	..	..	..	..	..	..	..
Vitamin C .. D.	164	172	180	188	196	204	212	220	228	236	244	252	260	268	276	284	292	300	308	316	324	..
F.	3	5	4	8	11	4	1	4	7	5	4	8	5	1	0	0	1	2	1	0	1	..
Calcium .. D.	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	..	..
F.	10	8	5	7	12	8	5	4	8	7	3	2	2	3	6	0	0	0	0	2	..	..
Phosphorus.. D	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	..	..	..	..	..	..	..
F.	5	11	8	11	6	8	6	2	1	2	0	1	0	0	1	..	..	..	..	..	..	..
Potassium .. D.	145	146	147	148	149	150	151	152	153	154	155	156	157	158	..	..	..	..	..	..	..	..
F.	12	9	9	21	3	12	6	4	5	5	0	4	2	1	..	..	..	..	..	..	..	..
Sodium .. D.	49	50	51	52	53	54	55	56	57	58	59	60	..	..	..	..	..	..	..	..	..	..
F.	10	18	10	8	5	5	5	4	1	3	1	1	..	..	..	..	..	..	..	..	..	..
Magnesium D.	13	14	15	16	17	18	19	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
F.	13	5	1	1	0	0	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Sulphur .. D	10	11	12	13	14	15	16	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
F.	24	8	9	1	1	0	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Iron .. D.	090	095	100	105	110	115	120	125	< 125	..	..	..	..	..	..	..	..	..	..	..	..	..
F.	7	1	2	2	4	0	0	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..

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particular season. Such results, obtained by spectrophotometric methods, have been published by Baumann et al. (1934), Beeson (1935), Booth et al. (1934), Gillam et al. (1936), Peterson et al. (1935), and Sutton and Kraus (1936). Sherman and Sherman (1937) give the mean vitamin A value of 86 samples of butter-fat as 50.60 ± 1.80 international units per gram, which differs only by three international units from the figure in Table I. Crawford et al. (1932) have determined the vitamin A activity of Australian butter-fat biologically, but because of the method used, the results are not directly comparable with those in this work. The frequency distribution table shows carotene to be a very variable constituent.

VITAMIN B_1.

Very few figures are to be found for the vitamin B_1 content of milk. Baker and Wright (1935) have published 23 international units per 100 ccs., which is four international units higher than the results obtained during this investigation, whilst Pyke (1937) found two samples contained 6 and 11 international units per 100 ml.

VITAMIN B_2.

Comparatively little work has been published on the vitamin B_2 content of milk. The figures .2 to .3 mgm. per 100 ccs., .1 mgm. per 100 ccs., and .176 to .26 mgm. per 100 ccs. have been obtained by Euler and Adler (1934), Kuhn et al. (1934), and Whitnah et al. (1937) respectively.

VITAMIN C.

The vitamin C content of milk has been investigated by many workers and appears to vary from $< .3$ (Levy and Fox, 1935) to 2.92 (Whitnah and Riddell, 1937) mgms. per 100 gms. 1.77 mgms. per 100 gms. which is slightly higher than the figure (1.56 mgms. per 100 gms.) given in Table I., is the mean of nineteen results obtained by Levy and Fox (1935), Whitnah and Riddell (1937), Ranganathan (1935), Fujita and Ebihara (1937), Meulemans and De Haas (1937), Rasmussen et al. (1936), Harris and Ray (1935), Correns (1937), Birch et al. (1933), Whitnah and Riddell (1936), Van Wijngaarden (1934), Kon and Watson (1937), Riddell et al. (1936), and Ferdinand (1936).

POTASSIUM, SODIUM, MAGNESIUM, AND SULPHUR.

Sherman (1936) gives .143 for the percentage of potassium in whole milk, Richmond (1930) .150, Crichton (1930) .168, and Trunz (1903) gives .136 and .149 per cent. For sodium Sherman gives .051 per cent., Richmond .037, Crichton .056, and Trunz gives .032 and .042 per cent. For the amount of magnesium in whole milk, Forbes et al. (1917) and Hart et al. (1909) each give .011 per cent., Sherman .012 per cent., Forbes et al. (1918) and Richmond .013 per cent., while Trunz gives the range .011 to .015 per cent. and .012 to .017 per cent. König (1904) gives .007 per cent. as the amount of sulphur in whole milk which has been ashed. (Approximately 72 per cent. of the sulphur in milk is lost in ashing). These figures are in good agreement with those obtained during this survey.

IRON.

Table IV. gives the iron content of milk according to various investigators :—

TABLE IV.—IRON CONTENT OF MILK.

Investigator.	Parts per Million.	Investigator.	Parts per Million.
Anselm (18[illegible]5)	·62– 84	Lesne et al (1930)	·95
Davies (1931)	1·5–2·4	Langstein (1911)	·21–·49
Edelstein and Csonka (1912) ..	·28– ·49	Macfarlane (1932)	·48–·68
Elvehjem (1926)	·35– ·36	Nottbohm and Dorr (1914) ..	·21–·19
Fendler et al. (1914)	2·8–8·4	Sherman (1936)	2
Friedjung (1901)	·84–1·82	Soxhlet (1912)	·18–·84
König (1896)	·35–4·69	Trunz (1903)	·22–·36
		This Investigation	·87

These figures vary considerably, but the figure for Australian milk is well within the common range.

CALCIUM.

Table V. shows that the mean figure for the calcium content of Australian milk agreees very well with figures obtained by overseas investigators.

TABLE V.—CALCIUM AND PHOSPHORUS CONTENT OF MILK.

Investigator.	Percentage Ca.	Percentage P.
Bunge (1901)	..	·077–·084
Burr and Witt (1935)	·083–·141	·071–·117
Cranfield et al. (1927)	·132	·102
Crichton (1930)	·119	·100
Davies and Provan (1928)	·124	·105
Forbes et al. (1917)	·103	·078
Forbes et al. (1918)	·117	·094
Golding et al. (1932)	·128	·107
Hart et al. (1909)	·084–·097	·076
Hutchison (1906)	..	·096
Katagama (1908)	·119	·094
Katagama (1908)	·127	·098
König (1904)	·112	·080
Meigs et al. (1926)	·100	·087
Meigs et al. (1926)	·132	·114
Richmond (1930)	·109	·096
Sheehy (1921)	..	·090
Sherman (1936)	·118	·093
Sommer and Hart (1926)	·135	·095
Sommer and Hart (1926)	·124	·113
Sommer and Hart (1926)	·142	·102
Sommer and Hart (1926)	·129	·103
Trunz (1903)	·137	·083–·103
Trunz (1903)	·128	·089–·100
Wellmann (1937)	·118–·146	·096–·155
This Investigation	·120	·082

PHOSPHORUS.

Table V. compares the mean figure for the phosphorus content of Australian milk with twenty-five figures obtained by overseas investigators. It will be seen that three of the overseas figures are slightly lower and one practically equal to the Australian figure, whilst twenty-one are definitely higher. Hence it was considered reasonable to conclude that the phosphorus content of the Australian samples was low.

However, it was within the normal range according to Bunge, Burr and Witt, Wellman (see Table V.) and the following investigators. Sommer (1929) gives the range .068 to .119 per cent., Cranfield et al. (1927) (673 samples) .076 to .135 per cent., although 80 per cent. of the samples fell between the range .092 to .111 per cent., and Crichton (1930) has found it to be .073 to .127 per cent. for 220 samples.

It was at first thought that this low figure may be due to the method of estimation, but this was checked against the well-established method of titrating the ash solution with uranium acetate using potassium ferrocyanide as external indicator when both methods gave results in good agreement.

It has been recognized for some decades that many Australian soils, particularly the superficial soils, are deficient in phosphorus and this has been indirectly proved by the remarkable results which follow the use in New South Wales, Victoria, and Tasmania of phosphatic fertilizers. In an article by Cherry (1907) Victorian clay soils were compared with American clay soils, the clay soils being chosen because they contained a higher percentage of phosphorus than other soils. It was shown that Victorian clay soils contained 63 parts of phosphoric acid per 100,000 parts, whereas American clay soils contained 207 parts per 100,000. The Mallee soils were even lower, containing only 47 parts per 100,000.

During a survey conducted by Henry and Benjamin (1933), it was found that of 56 soils analysed from the southern coastal belt of New South Wales, only eight samples contained as much as five parts per 100,000 of citrate soluble phosphoric acid, the remaining 48 samples averaging 2.3 parts per 100,000.

Many other papers have been written containing data on the low phosphorus content of Australian soils. In the eastern States there is the work of Taylor and Hooper (1938), Taylor and England (1929), Taylor and Penman (1930), and Taylor et al. (1933), whilst in Western Australia there is the work of Hosking and Burvill (1938) and Teakle (1929).

In unpublished work carried out in this laboratory it was found, after analysing eighty-six samples of soil collected from dairy farms situated on the northern coastal belt of New South Wales, that each of these soils was very low in phosphorus.

Many research workers have found that the mineral content of pastures, fodder crops, cereals, &c., is related to the mineral content of the soils on which they are grown. In order to investigate this, one hundred and fifty-six samples of pastures and fodder crops were collected from the eighty-six previously mentioned dairy farms at the time the milk and soil samples were collected. The phosphorus content of these samples, dried at 100°C., was determined by a method similar to that used for milk, and the results together with comparative figures by overseas investigators are given in Table VI.

TABLE VI.—PHOSPHORUS CONTENT OF FEEDS.

Feed.	Remarks.	P Per Cent.	Number of Samples.	Country.	Authority.
Mixed pasture	From fertilized land	·149	11	Australia ..	This investigation
,, ,,	From unimproved land	·178	46	,, ..	,, ,,
,, ,,	Good pasture ..	·46	..	New Zealand	Rigg and Askew (1929)
,, ,,	Poor hill pasture	·33	..	,, ,,	,, ,, ,,
,, ,,	From both manured and unmanured land	·335	40	England ..	Armstrong (1907)
,, ,,	From cultivated land	·32	24	England and Wales	Godden (1926)
,, ,,	From hill country	·26	35	Scotland ..	,, ,,
,, ,,	From natural pasture	·29	22	,, ..	Orr (1929)
,, ,,	From cultivated land	·32	24	,, ..	,, ,,
,, ,,	From poor hill country	·26	35	,, ..	,, ,,
,, ,,	.. .. ..	·29	12	Kenya ..	Orr and Holm (1931)
,, ,,	.. .. ..	·223	60	Mauritius ..	Lincoln (1937)
,, ,,	Natural Pasture	·303	86	United States	Newlander et al (1933)
,, ,,	,, ,,	·19	96	,, ,,	Archibald and Bennett (1933)
Fresh lucerne*	From fertilized land	·419	19	Australia ..	This investigation
,, ,, *	From unimproved land	·411	33	,, ..	,, ,,
,, ,, *	.. .. ..	·625	..	England ..	Woodman (1934)
,, ,,	.. .. ..	·457	..	South Africa	Fox and Wilson (—)
Green oats ..	From fertilized land	·520	4	Australia ..	This investigation
,, ,, ..	From unimproved land	·554	4	,, ..	,, ,,
Fresh sorghum	From fertilized land	·097	16	Australia ..	This investigation
,, ,,	From unimproved land	·089	5	,, ..	,, ,,
Bran ..	.. .. ..	·98	5	Australia ..	This investigation
,, ..	.. .. ..	1·32	..	America ..	Morrison (1936)
,, ..	.. .. ..	1·20	..	Indiana ..	Purdue Uni. Agr. Exp. Sta. (1938)
,, ..	.. .. ..	1·452	..	Europe ..	Heubner and Reeb (1908)

* Pre-budding period.

From this table it was seen that the phosphorus content of these pastures and fodder crops was very low. In some cases it was lower in crops grown on fertilized land than in crops grown on unfertilized land. This throws doubt on the testimonies of the farmers who supplied the information regarding fertilization of these lands, although it is possible that soils on which these crops grew may have been so low in phosphorus that the amount of fertilizer used restored but little of the deficiency.

The mean phosphorus content of the mixed milk from these eighty-six dairy farms was .081 per cent. This does not differ appreciably from the mean figure given in Table 1.

The question now arises as to whether the amount of phosphorus ingested by the cow determines the phosphorus content of milk. This question has been investigated by many workers and an excellent review of literature on the subject is included in a paper by Crichton (1930). On summing up the results of the various investigations a conclusion similar to that expressed by Forbes and Keith (1914) developed, namely, that the character of the feed may vary the phosphorus content of the milk, but only within normal limits.

Many of the milk samples collected during this survey were from herds which have been feeding for cow-generations on low phosphorus diets and often in localities where the conditions were so severe that cases of osteophagia were frequently observed (Henry and Benjamin, 1933). Where such conditions prevail, it seems reasonable to assume that this low phosphorus content of the milk can be due to no other cause than the low phosphorus content of Australian pastures and fodder crops, or indirectly to the low phosphorus content of Australian soils. This conclusion does not support that of Kincaid (1911), who analysed four milk samples from Victoria.

Correlations between the Vitamin and Mineral Constituents.

In a preliminary examination points were plotted for every value of each of the vitamins A, B_1, B_2, and C against the values for the mineral elements from the same milk sample. Twenty-eight graphs were thus obtained. These graphs were carefully examined, but in no instance could any correlation be detected between any two sets of results. Indeed, there was such a lack of correlation that it was considered unnecessary to apply any statistical treatment.

No correlation was found to exist between any of the vitamin and any of the mineral constituents of milk.

In view of the opinion expressed by Richmond (1930) that lecithin, because of its phosphorus content, may be an index of the vitamin content of milk, the lecithin content of thirty samples of milk was estimated by the method of Bordas and Raczkowski (1902) and the results examined as before, but again no correlation was detected. The mean figure for the lecithin content of these samples was .066 per cent.

A Correlation between the Phosphorus Content of Pasture and the Vitamin A Content of Milk.

Samples of feed as well as samples of milk were collected from most farms visited during this investigation, and during the examination of results, a most interesting correlation was found to exist between the phosphorus content of the pastures upon which certain herds grazed and the vitamin A content of the butter-fat from these herds.

On certain farms, which were comparatively large holdings, the herds were feeding on pasture alone. These farms (indicated by an asterisk in Table I.) were situated in the coastal dairying districts of New South Wales, the most southern being situated in the Richmond district and the majority of others in the Grafton district. According to information gathered from each farmer, these herds had been feeding in the same paddocks for several cow-generations, the pasture feed had never been supplemented with hand feed of any kind and the paddocks had never been artificially fertilized, so that the mineral and vitamin intake of these herds remained fairly constant throughout the year and from one year to another.

The specimens of pasture and milk were collected in late summer when pastures were poorest, but on each farm there was abundance of green grass on which the cattle could feed and, as will be seen later, the diet contained a large excess of carotene. The pasture samples, which were collected with the assistance of the farmers, were taken from many parts of the field and much care was taken to obtain a truly representative sample. The samples were not cut, but plucked by hand in order to simulate a cow's method of grazing, and herbage not eaten by the cow was not included in the sample. On most farms couch (*Cynodon* sp.) was the predominating grass, and on some farms this was the only pasture grass. Paspalum (*Paspalum* sp.) and various species of clover were also common, but native grasses other than couch constituted a very small portion of any pasture.

TABLE VII.

(*Phosphorus and protein expressed as percentage of pasture dried at* 100°*C.*)

I.U. Vitamin A /gm. of B.F.	Breed of Herd.	Percentage P in Pasture.	Percentage Protein in Pasture.	Carotene in Pasture mgms/kg.
58	Mixed	·217	10·12	180
38	,,	·085	9·04	190
60	Jersey	·171	10·51	240
40	Mixed	·098	6·72	200
45	,,	·133	6·56	200
56	,,	·202	10·73	190
59	,,	·186	9·05	180
66	,,	·209	10·62	180
55	Jersey	·187	12·43	200
72	Mixed	·325	13·15	210
44	,,	·101	10·20	250
38	,,	·116	9·72	190
55	,,	·169	9·54	190
40	Jersey	·147	10·89	200
46	Mixed	·096	9·05	200
46	,,	·111	10·21	190
45	,,	·186	12·20	180
52	Australian Illawarra Shorthorn	·132	8·45	200
44	Mixed	·124	8·35	180

Table VII. gives the amount of vitamin A per gram of butter-fat in the mixed milk from these herds and the percentages of phosphorus, protein, and carotene in the pastures upon which the herds were feeding. It will be seen that a direct correlation exists between the vitamin A content of the butter-fat and the phosphorus content of the pasture, the coefficient of correlation being .97.

The carotene content of the pastures was estimated by a method similar to that given by Bolin and Khalapur (1938) and it will be seen from Table VII. that the poorest pasture contained 180 mgms. of carotene per kilogram or approximately 288,000 international units of vitamin A per kilogram (Fixsen and Roscoe, 1937-38). According to the work of Fraps et al. (1937) and Guilbert and Hart (1935), cows on these pastures were receiving a large daily excess of vitamin A.

Hence it would appear that the vitamin A content of butter-fat is influenced in some way by the phosphorus as well as the carotene ingested by the cow as suggested by Professor Rosedale. However, there may be other underlying factors such as the stage of growth of the pasture, for it will be seen that there is also a considerable degree of correlation between the protein and phosphorus content of the pastures. Unfortunately, most of the milk samples were from mixed herds, and because of this the table loses some of its value. An interesting piece of confirmatory evidence is given by the following fact, however. The mean vitamin A content of five samples of mixed milk from cows feeding on pasture but receiving in addition a daily ration of bran (a rich source of phosphorus) was two international units higher than the mean figure for the results given in Table VII.

No similar correlation was found to exist between any of the other vitamins and the phosphorus or protein intake of the herd.

Summary.

Fat, vitamin A, carotene, vitamin B_1, vitamin B_2, vitamin C, calcium, phosphorus, potassium, sodium, magnesium, sulphur, and iron have been estimated in 168 samples of mixed milk. Methods and results have been presented. The mean results of all analyses appear in the following table:—

Specific gravity	1·0318	Calcium ..	·120 per cent.
Total solids	13·50 per cent. ..	Phosphorus ..	·082 per cent.
Solids-not-fat	8·99 per cent. ..	Lecithin ..	·066 per cent.
Fat	4·51 per cent. ..	Potassium ..	·144 per cent.
Carotene in Butter-Fat ..	·00104 per cent. ..	Sodium ..	·048 per cent.
Vitamin A in Butter-Fat ..	47·6 I.U./gm. ..	Magnesium ..	·012 per cent.
Vitamin B_1	18·8 I.U./100 ccs. ..	Sulphur ..	·009 per cent.
Vitamin B_2	·17 mgm/100 ccs...	Iron ..	·000087 per cent.
Vitamin C	·00156 per cent. ..		

By comparing the results with overseas figures it has been shown that the phosphorus content of the Australian milk, although within normal limits, was low. It has also been shown that no correlations exist between the vitamin and mineral constituents of milk. Subject to certain conditions, a correlation was discovered between the vitamin A content of butter-fat and the phosphorus content of the pasture.

Acknowledgments.

I wish to acknowledge with gratitude the assistance which I have received from Professor Harvey Sutton, Director of the School of Public Health and Tropical Medicine in Sydney, in arranging with the Milk Board of New South Wales and numerous butter factory managers for the collection of many of the samples, and from Dr. F. W. Clements of the Australian Institute of Anatomy, in giving me every possible opportunity to do this work.

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